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Filed PCT/PTO 19 AUG 2002

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Menke, Anette
Azzeah, Maysa

<120> Stable Recombinant Influenza Viruses Free of Helper
Viruses

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| gcattgtctg | tatttgacag | gagccactcc | atttctgcat | agaatgaaga | ccctgatctt | | 1440 |
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| ttgtcaatcc | cacctgatcc | tctgaggatt | tgtcgcaatg | cctcttcatt | aacaaacttc | | 1560 |
| cccgggtaac | aaacatcatt | tccttctcgt | ctctcgatta | ttagatcagc | tgaataattct | | 1620 |
| agaaattggt | cgcattgagg | tgggtccggt | atgggtcccta | acagtcgcca | ttggccaaga | | 1680 |
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| gttgcatgga | caacttctac | tcctctctca | gtgagtgtgt | ttactttggt | gccatttgat | | 1800 |
| acagcatgat | gtccaagaca | aattttgtct | gcatttggtg | ggatgactgc | cgcaagggcg | | 1860 |
| aaaaccagga | tttgagtgtt | cattttggtg | cctacaatat | gtttttcgtc | tcagccaatc | | 1920 |
| cctggtacaa | tatgtttttc | gtctcagcca | atcctggtac | gatgtggatg | tcactcagtg | | 1980 |
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<210> 4

<211> 1146

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: vHM81

<400> 4

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| aagggaacaa | aagctggagc | tccaccgcgg | tggcggccgc | tctagcatat | gtttttcgtc | 120 |
| tcagccaatc | cctctagcat | atgtttttcg | tctcagccaa | tccctctaga | agcttcgtac | 180 |
| gcattgctaa | ataagctgaa | acgagaaagt | tcttatctct | tgctccactt | caagcggtag | 240 |
| ttgtaaggct | tgataaaatg | ttatttggtc | aaaactattc | tctgttatct | tcaatctatg | 300 |
| tctcacttct | tcaattaacc | atcttatttc | ttcaaatctc | tgactcaatt | gttctcgcca | 360 |
| ttttccgttt | ctgcttttga | gggagtggag | gtcccccatt | ctcattactg | cttctccaag | 420 |
| cgaatctctg | tatatgtttc | gagactcgaa | ctgtgttatc | attccattca | agtcctccga | 480 |
| tgaggacccc | aattgcattt | ttgacatcct | catcagtatg | tcctggaaga | gaaggcaatg | 540 |
| gtgaaatttc | gccgacaatt | gctccctcat | cggttaaagc | ccttaaatag | atgagagttt | 600 |
| ccagccgatc | gaaaatcaca | ctgaagtgtg | ctttcagtat | gatgttcttc | cccatgatcg | 660 |
| cctggtccat | tctgatgcaa | agggagcctg | ccactttctg | tttgggcatg | agcatgaacc | 720 |
| agtcccttga | catctcttca | agagtcatgt | cagttaggta | gcgtgtagca | ggtacagagg | 780 |
| caatgggtcat | tttaagtgcc | tcacgcgatt | cgctctccag | aatccgctcc | actatctgct | 840 |
| ttccaacacg | agtagctgtg | tcgatgtcca | gaccaagagt | gctgcctctt | ccccctcagg | 900 |
| acttctgatc | tcggcgaagt | cgggtcaagga | atggggcatc | acccatttct | tgggtctgcaa | 960 |
| atcgtttgcg | gacatgccaa | agaaagcagt | ctacctgaaa | gcttgacaca | gtgttggaat | 1020 |
| ccattatggt | acctacaata | tgtttttcgt | ctcagccaat | ccctgggtaca | atatgttttt | 1080 |
| cgtctcagcc | aatcctggta | cgatgtggat | gtcactcagt | gatgtgattat | ctaccctgct | 1140 |
| tttgct | | | | | | 1146 |

<210> 5

<211> 5860

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: pAM424

<400> 5

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|------------|------------|------------|------------|------------|------------|----|

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| | | | | | | |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| aattcatcga | aatgaccgac | caagcgacgc | ccaacctgcc | atcacgagat | ttcgattcca | 120 |
| ccgccgcctt | ctatgaaagg | ttgggcttcg | gaatcgtttt | ccgggacgcc | ggctggatga | 180 |
| tcctccagcg | cggggatctc | atgctggagt | tcttcgccc | ccccgggctc | gatccccctg | 240 |
| cgagttgggt | cagctgctgc | ctgaggctgg | acgacctcgc | ggagttctac | cggcagtgca | 300 |
| aatccgctcg | catccaggaa | accagcagcg | gctatccgcg | catccatgcc | cccgaactgc | 360 |
| aggagtgggg | aggcacgatg | gccgctttgg | tcccggatct | ttgtgaagga | accttacttc | 420 |
| tgtgggtgta | cataattgga | caaaactacct | acagagattt | aaagctctaa | ggtaaataata | 480 |
| aaatttttaa | gtgtataatg | tgttaaacta | ctgattctaa | ttgtttgtgt | atttttagatt | 540 |
| ccaacctatg | gaactgatga | atgggagcag | tggtggaatg | cctttaatga | ggaaaacctg | 600 |
| ttttgctcag | aagaaatgcc | atctagtgat | gatgaggcta | ctgctgactc | tcaacattct | 660 |
| actcctccaa | aaaagaagag | aaaggtagaa | gaccccaagg | actttccttc | agaattgcta | 720 |
| agttttttga | gtcatgctgt | gtttagtaat | agaactcttg | cttgctttgc | tatttacacc | 780 |
| acaaaggaaa | aagctgcact | gctatacaag | aaaattatgg | aaaaatattc | tgtaaccttt | 840 |
| ataagtaggc | ataacagtta | taatcataac | atactgtttt | ttcttactcc | acacaggcat | 900 |
| agagtgtctg | ctattaataa | ctatgctcaa | aaattgtgta | ccttttagctt | tttaatttgt | 960 |
| aaaggggtta | ataaggaata | tttgatgtat | agtgccttga | ctagagatca | taatcagcca | 1020 |
| taccacattt | gtagaggttt | tacttgcttt | aaaaaacctc | ccacacctcc | ccctgaacct | 1080 |
| gaaacataaa | atgaatgcaa | ttgttgttgt | taacttgttt | attgcagctt | ataatggtta | 1140 |
| caaataaaagc | aatagcatca | caaatttcac | aaataaagca | tttttttcac | tgcattctag | 1200 |
| ttgtgggttg | tccaaactca | tcaatgtatc | ttatcatgtc | tggatcccca | ggaagctcct | 1260 |
| ctgtgtcctc | ataaacctca | acctcctcta | cttgagagga | cattccaatc | ataggctgcc | 1320 |
| catccacctt | ctgtgtcctc | ctgttaatta | ggctacttaa | caaaaaggaa | attgggtagg | 1380 |
| ggtttttcac | agaccgcttt | ctaagggtaa | ttttaaaata | tctgggaagt | cccttccact | 1440 |
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| ccaaaacagc | cttgtgggtca | gtgttcattc | gctgactgtc | aactgtagca | ttttttgggg | 1740 |
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| gttccccacc | aacagcaaaa | aaatgaaaaat | ttgaccttg | aatgggtttt | ccagcaccat | 1860 |
| tttcatgagt | tttttgtgtc | cctgaatgca | agtttaacat | agcagttacc | ccaataacct | 1920 |
| cagttttaaac | agtaacagct | tcccacatca | aaatatttcc | acaggttaag | tcctcattta | 1980 |
| aattaggcaa | aggaattctt | gaagacgaaa | gggcctcgtg | atacgcttat | ttttataggt | 2040 |
| taatgtcatg | ataataatgg | tttcttagac | gtcaggtggc | acttttcggg | gaaatgtgag | 2100 |
| cggaacccct | atttgtttat | ttttctaaat | acattcaaat | atgtatccgc | tcattgagaca | 2160 |
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| agactggatg | gaggcgggata | aagttgcagg | tgagcgtggg | cgctcgggcc | ttccggctgg | 2880 |
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<211> 4610

<212> DNA

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| ccccccccc | ggcgcggaac | ggcggggcca | ctctggactc | tttttttttt | tttttttttt | 1920 |
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| accaccgcta | ccagcgggtg | tttgtttgcc | ggatcaagag | ctaccaactc | tttttccgaa | 3600 |
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| aggccaccac | ttcaagaact | ctgtagcacc | gcctacatac | ctcgtctctg | taatcctgtt | 3720 |
| accagtggct | gctgccagtg | gcgataagtc | gtgtcttacc | gggttggaact | caagacgata | 3780 |
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| gcttcccga | gggagaaaag | cggacaggta | tccggttaagc | ggcagggtcg | gaacaggaga | 3960 |
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| ccacctctga | cttgagcgtc | gattttttgt | atgctcgtca | ggggggcgga | gcctatggaa | 4080 |
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| gttctttcct | gcgttatccc | ctgattcatt | aatgcaggtc | acgatccttt | ctggcgagtc | 4200 |
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| tgtgtggctg | cgatgggtgg | gtttttgggg | acaggtgtcc | gtgtccgtgt | cgcgcgctcg | 4500 |
| ctgggcgggc | ggcgtggtcg | gtgacgcgac | ctcccggccc | cgggggagggt | atatctttcg | 4560 |
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<211> 3558

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PHL2583

<400> 7

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| gcagtactgt | tgtaattcat | taagcattct | gccgacatgg | aagccatcac | agacggcatg | 120 |
| atgaacctga | atcgccagcg | gcatcagcac | cttgctgcct | tgctataat | atgtgccat | 180 |
| ggtgaaaacg | ggggcgaaga | agttgtccat | attggccacg | tttaaatcaa | aactggtgaa | 240 |
| actcaccag | ggattggctg | agacgaaaaa | catattctca | ataaacccct | tagggaaata | 300 |
| ggccagggtt | tcaccgtaac | acgccacatc | ttgcgaatat | atgtgtagaa | actgccgga | 360 |
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| cttatttttc | tttacggctc | ttaaaaaggg | cgtaatatcc | agctgaacgg | tctggttata | 600 |
| ggtacattga | gcaactgact | gaaatgcctc | aaaatgttct | ttacgatgcc | attgggatat | 660 |
| atcaacggtg | gtatatccag | tgattttttt | ctccatgatt | atggccatta | cccttgtttc | 720 |
| tactcccccc | caacttcgga | ggtcgaccag | tactccgggc | gaaactttgt | tttttttttt | 780 |
| tcccccgatg | ctggaggctc | accagatgtc | cgaaagtgtc | ccccccccc | ccccccccc | 840 |
| gcgcggaacg | gcggggccac | tctggactct | tttttttttt | tttttttttt | tttttgggga | 900 |
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| tcagaacgca | gaagcggctc | gataaaacag | aatttgccctg | gcggcagtag | cgcggtggtc | 1020 |
| ccacctgacc | ccatgccgaa | ctcagaagtg | aaacgccgta | gcgccgatgg | tagtgtgggg | 1080 |
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| agactggggc | tttcgtttta | tctgtttgtt | gtcgggtgaac | gctctcctga | gtaggacaaa | 1200 |
| tccgcccggg | gcggatttga | acgttgcgaa | gcaacggccc | ggagggtggc | gggcaggacg | 1260 |
| cccgccataa | actgccaggc | atcaaattaa | gcagaaggcc | atcctgacgg | atggcctttt | 1320 |
| tgcgtttcta | caaactcttt | tgtttttttt | tctaaatata | ttcaaataatg | tatccgctca | 1380 |
| tgagacaata | accctgataa | atgcttcaat | aatattgaaa | aagggaagagt | atgagtattc | 1440 |
| aacattttcc | tgctgccttt | attccctttt | ttgcggcatt | ttgccttctc | gtttttgctc | 1500 |
| acccagaaaac | gctggtgaaa | gtaaaagatg | ctgaagatca | gttgggtgca | cgagtggggt | 1560 |
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| ccgggcaaga | gcaactcggg | cgccgcatac | actattctca | gaatgacttg | gttgagtact | 1740 |
| caccagtcac | agaaaagcat | cttacggatg | gcatgacagt | aagagaatta | tgcatgtctg | 1800 |
| ccataaccat | gagtgataac | actgcggcca | acttacttct | gacaacgatc | ggaggaccga | 1860 |
| aggagctaac | cgcttttttg | cacaacatgg | gggatcatgt | aactcgcctt | gatcgttggg | 1920 |
| aaccggagct | gaatgaagcc | ataccaaacg | acgagcgtga | caccacgatg | cctgtagcaa | 1980 |
| tggcaacaac | gttgcgcaaa | ctattaactg | gcgaactact | tactctagct | tccgggcaac | 2040 |
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| cggtctggct | gtttattgct | gataaatctg | gagccgggtga | gcgtgggtct | cgcggtatca | 2160 |
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| gtcaggcaac | tatggatgaa | cgaaatagac | agatcgctga | gatagggtgc | tcactgatta | 2280 |
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| atttttaatt | taaaaggatc | taggtgaaga | tcctttttga | taatctcatg | accaaatacc | 2400 |
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<211> 4343

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: pHL2989

<400> 8

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<210> 9

<211> 3888

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PHL1920

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| ggcgcgacct | ctcgggccc | acgcgcgctc | agggagcgct | ctccgactcc | gcacggggac | 1620 |
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| cctgttccga | ccctgccgct | taccggatac | ctgtccgcct | ttctcccttc | gggaagcgtg | 1920 |
| gcgctttctc | atagctcagc | ctgtaggtat | ctcagttcgg | tgtaggtcgt | tcgctccaag | 1980 |
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| cgagttacat | gatcccccat | gttggtgcaa | aaagcggtta | gctccttcgg | tcctccgatc | 2940 |
| gttgtcagaa | gtaagtggc | cgagtggtta | tcactcatgg | ttatggcagc | actgcataat | 3000 |
| tctcttactg | tcatgccatc | cgtaagatgc | ttttctgtga | ctgggtgagta | ctcaaccaag | 3060 |
| tcattctgag | aatagtgtat | gcggcgaccg | agttgtctct | gcccggcgctc | aacacgggat | 3120 |
| aataccgcgc | cacatagcag | aactttaaaa | gtgtctcatca | ttggaaaacg | ttcttcgggg | 3180 |
| cgaaaactct | caaggatctt | accgctgttg | agatccagtt | cgatgtaacc | cactcgtgca | 3240 |
| cccaactgat | cttcagcatc | ttttactttc | accagcggtt | ctgggtgagc | aaaaacagga | 3300 |
| aggcaaaatg | ccgcaaaaaa | gggaataagg | gcgacacgga | aatgttgaat | actcatactc | 3360 |
| ttcctttttc | aatattattg | aagcatttat | cagggttatt | gtctcatgag | cggatacata | 3420 |
| tttgaatgta | tttagaaaaa | ttaaacaagg | agtttgtaga | aacgcaaaaa | ggccatccgt | 3480 |
| caggatggcc | ttctgcttaa | tttgatgcct | ggcagtttat | ggcgggctgc | ctgcccgcga | 3540 |
| ccctccgggc | cgttgcttcg | caacgttcaa | atccgcctcc | ggcggatttg | tcctactcag | 3600 |
| gagagcggtc | accgacaaac | aacagataaa | acgaaaggcc | cagtctttcg | actgagcctt | 3660 |
| tcgttttatt | tgatgcctgg | cagttcccta | ctctcgcatg | gggagacccc | acactaccat | 3720 |
| cggcgctacg | gcgttttact | tctgagttcg | gcatggggtc | aggtgggacc | accgcgctac | 3780 |
| tgccgcccag | caaattctgt | tttatcagac | cgcttctgcg | ttctgattta | atctgtatca | 3840 |
| ggctgaaaaa | cttctctcat | ccgcaaaaac | agaagctagc | ggccgatc | | 3888 |

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 <211> 12
 <212> RNA
 <213> Influenza A virus

<400> 10
 ccugcuuuug cu

12

<210> 11
 <211> 12
 <212> RNA
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<220>
 <221> variation
 <222> 1...2
 <223> a or g or c or t/u at position 1 or 2

<400> 11
 nnygcuucug cu

12

~7048421

<210> 12
<211> 12
<212> RNA
<213> Influenza C virus

<400> 12
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12

<210> 13
<211> 12
<212> RNA
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<220>
<223> Description of Artificial Sequence: Modified
influenza A 3' sequence (pHL1104 and 1920)

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12

<210> 14
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<212> RNA
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<220>
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influenza A 3' sequence (pHL1948)

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12

<210> 15
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<220>
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influenza A 5' sequence (pHL1920)

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13

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13

<210> 17
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<212> RNA
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~7048421

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<400> 17
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<210> 18
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<400> 18
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<210> 19
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 <222> 14...16
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<400> 19
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<210> 20
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 <213> Artificial Sequence

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 <222> 14...16
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 Description of Artificial Sequence: Modified
 influenza A 5'-sequence (pHL1920)

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<210> 21
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 <212> RNA
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<220>
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 or 14 or 15 or 16

~7048421

<400> 21
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21

<210> 22
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<212> RNA
<213> Artificial Sequence

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influenza C 5' sequence

<400> 22
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19

<210> 23
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<220>
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Description of Artificial Sequence: Modified
influenza A 3' sequence (pHL1104 and 1920)

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15

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<212> RNA
<213> Artificial Sequence

<220>
<221> variation
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Description of Artificial Sequence: Modified
influenza A 3' sequence (pHL1948)

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15

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<212> RNA
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<220>
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or 3 or 4 or 5
Description of Artificial Sequence: Modified
influenza B 3' sequence

<400> 25

~7048421

15

nnnnnyguuu cuacu

<210> 26

<211> 14

<212> RNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Modified
influenza C 3' sequence

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14